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14. ABSTRACT

We hypothesize that excessive cap-dependent translation is a causative factor in autism spectrum disorder (ASD). To test this hypothesis, we have been studying transgenic mice that overexpress eIF4E have been testing the following specific aims: 1) to determine whether eIF4E transgenic mice display behaviors consistent with ASD, 2) to determine whether ASD-like behaviors displayed by eIF4E transgenic mice can be reversed by novel cap-dependent translation inhibitors, and 3) to determine whether eIF4E transgenic mice display cellular and molecular abnormalities due to excessive cap-dependent translation. mice. Our studies will provide information concerning whether overexpression of eIF4E is a *biological risk factor* for ASD. Our studies also will provide important information concerning the role of upregulated cap-dependent translation in ASD, and could link ASD mechanistically at the level of cap-dependent translational control to fragile X syndrome (FXS), tuberous sclerosis complex (TSC), and autistic patients with *PTEN* and *EIF4E* mutations. Moreover, the results of these studies would provide information for the design and use of compounds to *therapeutically target* eIF4E-eIF4G interactions and eIF4A for treating patients with ASD.

15. SUBJECT TERMS
autism spectrum disorder, cap-dependent translaiton, eIF4E, repetitive behaviors, perseverative behaviors, social behaviors

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Introduction

We hypothesize that excessive cap-dependent translation is a causative factor in autism spectrum disorder (ASD). To test this hypothesis, we have been studying transgenic mice that overexpress eIF4E have been testing the following specific aims: **1) to determine whether eIF4E transgenic mice display behaviors consistent with ASD, 2) to determine whether ASD-like behaviors displayed by eIF4E transgenic mice can be reversed by novel cap-dependent translation inhibitors, and 3) to determine whether eIF4E transgenic mice display cellular and molecular abnormalities due to excessive cap-dependent translation.** We have been determining whether eIF4E transgenic mice display behaviors consistent with ASD by examining their social behaviors, anxiety-related behaviors, perseverative/repetitive behaviors, and their responses to sensory stimuli. In previous studies we have used a novel compound termed 4EGI-1 that selectively disrupts interactions between translation factors eIF4E and eIF4G to inhibit translation in the brain. Therefore, we have been determining whether 4EGI-1 reverses ASD-like behaviors displayed by eIF4E transgenic mice. Moreover, because eIF4E/eIF4G interactions promote the helicase activity of eIF4A to stimulate cap-dependent translation, we will determine whether the eIF4A inhibitor hippuristanol can reverse ASD-like behaviors displayed by the eIF4E transgenic mice. Finally, we have been conducting studies to determine whether eIF4E transgenic mice have increased translation, altered neuronal morphology, and altered synaptic plasticity due to excessive cap-dependent translation. Our studies will provide information concerning whether overexpression of eIF4E is a ***biological risk factor*** for ASD. Our studies also will provide important information concerning the role of upregulated cap-dependent translation in ASD, and could link ASD mechanistically at the level of cap-dependent translational control to fragile X syndrome (FXS), tuberous sclerosis complex (TSC), and autistic patients with *PTEN* and *EIF4E* mutations. Moreover, the results of these studies would provide information for the design and use of compounds to ***therapeutically target*** eIF4E-eIF4G interactions and eIF4A for treating patients with ASD.

Body

Herein I will describe the research accomplishments associated with each task that was outlined in the approved Statement of Work.

The first task in the Statement of Work was to obtain regulatory approval for the use of mice by New York University (NYU) IACUC Committee and USAMRMC Office of Research Protections. The animal protocol was approved the NYU IACUC and Committee as well as the USAMRMC Office of Research Protections.

The second task in the Statement of Work was to determine whether the eIF4E transgenic mice exhibit behaviors consistent with ASD. This included subtask 1, which was to measure the social behaviors of the eIF4E transgenic mice and subtask 2, which was to measure anxiety-related behaviors, perseverative/ repetitive behaviors, and sensorimotor gating of the eIF4E transgenic mice. The results of these studies have been quite exciting.

Perseverative and repetitive behaviors are one of the domains required for the diagnosis of ASD (Lewis et al., 2007). These behaviors include stereotypies (purposeless repetitive movements and activities) as well as cognitive inflexibility (inability to disengage from a previously learned behavior and adopt a new behavioral strategy). We employed a marble burying test to quantify repetitive digging behavior (Thomas et al., 2009) and found that the eIF4E transgenic mice buried significantly more marbles compared to their wild-type littermates (Fig. 1A). We also examined self-grooming, which is a stereotypic repetitive behavior that has been observed in other mouse models of ASD (Peca et al., 2011; McFarlane et al., 2008) and discovered that the eIF4E transgenic mice exhibited increased self-grooming (Fig. 1B). We next evaluated whether eIF4E transgenic mice exhibited cognitive inflexibility

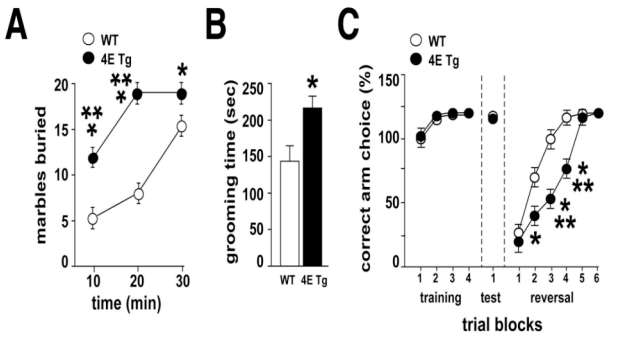

Figure 1. (**A**) eIF4E transgenic mice exhibit enhanced repetitive behavior in the marble burying test. Number of marbles buried in 10 min intervals. n=21-22 mice/genotype. ***p<0.001 and *p<0.05 vs WT, repeated measures ANOVA [time X genotype, $F_{(2,46)}$=31.62, p< 0.001] followed by Bonferroni-Dunn test. (**B**) eIF4E transgenic mice show increased self-grooming. n=12 mice/genotype. *p<0.05 vs WT, Student's t-test. (**C**) eIF4E transgenic mice exhibit impaired cognitive flexibility in the Y-maze reversal task. Percent correct arm choice by trial block number during training, test and reversal phases of the Y-maze. n=21-22 mice/genotype. *p<0.05 and ***p<0.001 vs WT, repeated measures ANOVA [time X genotype, $F_{(5,138)}$=16.74, p< 0.001] followed by Bonferroni-Dunn test.

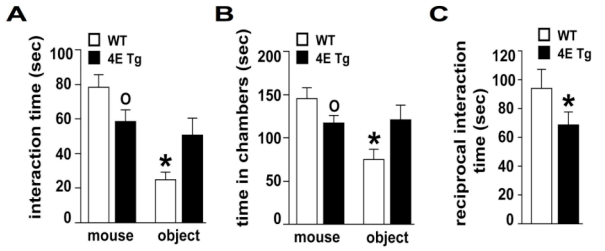

Figure 2. (**A**, **B**) eIF4E transgenic mice exhibit abnormalities in social behavior. Time spent either interacting with a stranger mouse and an object (**A**) or time spent in the chambers (**B**). n=6 mice/genotype. *p< 0.05 and °p< 0.05 vs. WT, repeated measures ANOVA [**A**: stimulus X genotype, $F_{(1,10)}$=6.04, p< 0.05; **B**: stimulus X genotype, $F_{(1,10)}$=6.12, p< 0.05] followed by Bonferroni-Dunn test. (**C**) eIF4E transgenic mice exhibit impaired reciprocal social interactions. Time spent interacting with a stranger mouse. n=6 mice/genotype. *p<0.05 vs WT controls, Student's t-test. All data are shown as mean ± SEM.

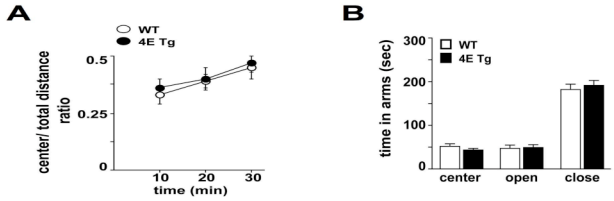

Figure 3. (**A**) Open field test. Ratio center/total distance. (**B**) Elevated plus maze test. Time spent in the arms and in the center of the maze. n.s., two-way ANOVA.

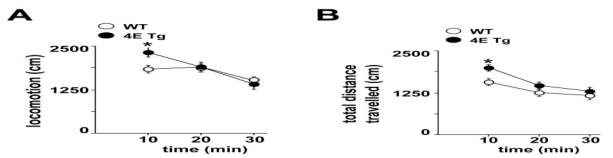

Figure 4. (**A**) Novelty-induced locomotor activity shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, F(5,100)=3.69, p<0.01] followed by Bonferroni-Dunn test. Open field test. (**B**) Total distance shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, F(5,100)=3.82, p<0.01] followed by Bonferroni-Dunn test.

by examining choice arm reversal in a water-based Y-maze task (Hoeffer et al., 2008). eIF4E transgenic mice showed intact learning abilities during the acquisition phase of the task and normal memory for the escape arm when tested 24 hours later. However, when the position of the escape arm was changed in the reversal phase of the task, eIF4E transgenic mice required significantly more trials to satisfy the same success criterion compared to their wild-type littermates (Fig. 1C). Taken together, these experiments suggest that increased eIF4E expression and consequently, exaggerated cap-dependent protein synthesis, results in repetitive and perseverative behaviors.

Abnormalities in social interaction skills are another behavioral defect displayed by individuals with ASD (Rapin and Tuchman, 2008). Thus, we also tested social behavior in eIF4E transgenic mice with two well-established behavioral paradigms, the three-chamber arena and the reciprocal social interaction task (Moy et al., 2004). eIF4E transgenic mice exhibited reduced preference for a nonspecific stranger as indicated by an equal amount of time spent interacting with the mouse stranger and a novel object (Fig. 2A). Similarly, the eIF4E transgenic mice displayed a reduced preference for the chamber where the stranger mouse was located (Fig. 2B). Moreover, eIF4E transgenic mice also exhibited diminished reciprocal interactions with a freely moving stranger mouse (Fig. 2C), further supporting deficits in social behavior. The deficits in social behavior of the eIF4E transgenic mice are unlikely to be caused by a generalized increased anxiety since the mice did not display anxiety-like traits when tested in other paradigms (i.e. center/total distance ratio in the open field test and elevated plus maze, (Fig. 3A and 3B). Moreover, the eIF4E transgenic mice exhibited a mild hyperactivity (first 10 min of novelty and open field tests, Fig. 4A and 4B) but no impairments in motor coordination, motor learning and sensorimotor gating abilities (Fig. 5A, 5B, and 5C). All together, the behavioral analysis of the eIF4E transgenic mice indicates that increased cap-dependent protein synthesis in the brain results in a distinct pattern of behavioral abnormalities consistent with ASD.

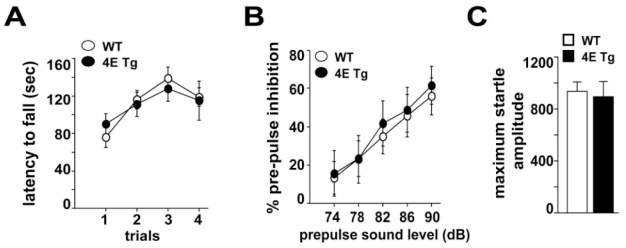

Figure 5. (**A**) Accelerating rotarod test. Latency to fall from the accelerating rod (sec) is shown for four test trials. n.s., repeated measure ANOVA. (**B, C**) Prepulse inhibition (PPI) of the acoustic startle response is represented as % of PPI of the startle response (**A**). n.s., repeated measures ANOVA. Acoustic startle response is expressed as maximum startle amplitude to the 120-dB stimulus (**L**). n.s.,Student's *t*-test. In all the experiments n=12-13 mice/genotype. All data are shown as mean ± SEM.

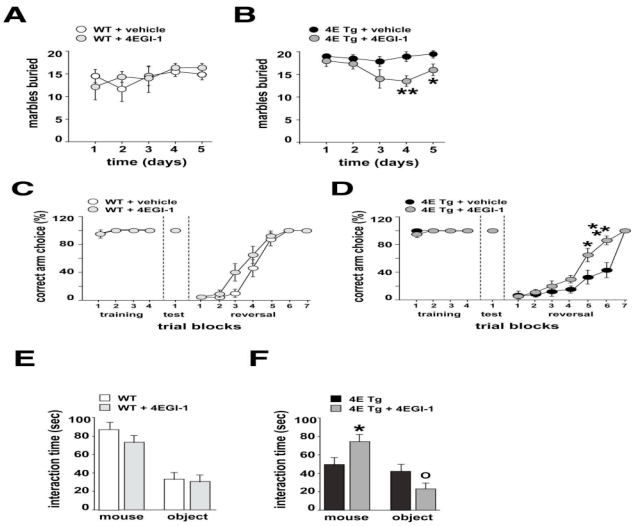

Figure 6. (**A, B**) 4EGI-1 reduces the marble-burying behavior of eIF4E transgenic mice. n=6 mice/genotype/treatment. ***p*< 0.01, **p*< 0.05 vs. 4E Tg + vehicle, two-way repeated measures ANOVA [treatment X genotype, $F_{(1,20)}$= 4.21, *p*< 0.05] followed by Bonferroni-Dunn test. (**C, D**) 4EGI-1 improves cognitive flexibility of eIF4E transgenic mice in the Y-maze test. Percent correct arm choice by trial block number during training, test and reversal phases of the Y-maze test. n=6-7 mice/genotype/treatment. ***p*< 0.01, **p*< 0.05 vs. 4E Tg + vehicle, two-way repeated measures ANOVA [treatment X genotype, $F_{(1,21)}$=4.61, *p*< 0.05] followed by Bonferroni-Dunn test. (**E, F**) 4EGI-1 improves social behavior of eIF4E transgenic mice in the three-chamber arena test. n=6 mice/genotype/treatment.**p*< 0.05 and °*p*< 0.05 vs. 4E Tg + vehicle, two-way repeated measures ANOVA [treatment X genotype, $F_{(1,20)}$=6.26, *p*< 0.05] followed by Bonferroni-Dunn test.

The third task in the Statement of Work was to determine whether the ASD-like behaviors displayed eIF4E transgenic mice could be reversed by novel cap-dependent translation inhibitors. Subtask 1 was to determine whether ICV infusion of 4EGI-1 could reverse ASD-like behaviors exhibited by eIF4E transgenic mice. Subtask 2 was to determine whether ICV infusion of hippuristanol can reverse ASD-like behaviors by eIF4E transgenic mice.

We have largely completed the experiments in first subtask. We employed a subthreshold dose of 4EGI-1 previously described in our laboratory (Hoeffer et al., 2011) to normalize the behavioral abnormalities in eIF4E transgenic mice without impairing their wild-type littermates. We infused either 4EGI-1 or vehicle directly into the lateral ventricle of cannulated eIF4E transgenic mice and their wild-type littermates. eIF4E transgenic mice treated with 4EGI-1 exhibited a decrease in repetitive behavior in the marble burying task starting on day four and persisted throughout day five, whereas the wild-type mice treated with 4EGI-1 behaved similarly to the vehicle-treated wild-type mice (Fig. 6A and 6B). We then tested the ability of 4EGI-1 to correct the behavioral inflexibility displayed by eIF4E transgenic mice in the Y-maze. We found that blockade of eIF4E/eIF4G interactions with 4EGI-1 significantly improved the performance of eIF4E transgenic mice by decreasing the number of trials required to reach the success criterion in the reversal phase of the task (Fig. 6C and 6D). These findings indicate that chronic treatment of eIF4E transgenic mice with 4EGI-1 reverses their repetitive and perseverative behaviors.

Finally, we determined whether infusions of 4EGI-1 also rescued the social behavior deficits displayed by the eIF4E transgenic mice. We found that eIF4E transgenic mice infused with 4EGI-1 for four days exhibited a preference for a non-specific stranger as indicated by an increased amount of time spent in interacting with the stranger mouse over the novel object (Fig. 6E and 6F). This result suggests that chronic treatment with 4EGI-1 also corrects social behavior deficits displayed by eIF4E transgenic mice.

We have not begun the second subtask to determine whether ICV infusion of hippuristanol can reverse ASD-like behaviors by eIF4E transgenic mice. We plan on beginning these experiments early in the second year of funding.

The fourth task in the Statement of Work was to determine whether the eIF4E transgenic mice

display cellular and molecular abnormalities due to excessive cap-dependent translation. Subtask 1 is to determine whether cap-dependent protein synthesis is increased in the brains of eIF4E transgenic mice. Subtask 2 is to determine whether eIF4E transgenic mice exhibit altered dendritic spine morphology and if so, whether 4EGI-1 and hippuristanol reverse the alterations. Subtask 3 is to determine whether eIF4E transgenic exhibit abnormal protein synthesis-dependent synaptic plasticity and if so, whether 4EGI-1 and hippuristanol reverse the abnormalities.

Although we have begun a number of these studies, the data are for the most part too preliminary to report at this time. We will continue these studies and complete them in years two and three of funding.

The fifth task is data analysis and reporting. As evidenced in this report, we have been analyzing the data throughout the performance of the experiments. Final data analysis and summaries will be prepared for reporting at the end of the performance period in year three of funding.

Please note that a detailed description of the behavioral tests is provided in the Appendix.

Key Research Accomplishments

- Demonstration that eIF4E transgenic mice display repetitive and perseverative behaviors that are consistent with ASD.

- Demonstration that eIF4E transgenic mice display impaired social behaviors that are consistent with ASD.

- Demonstration that repetitive/perseverative behaviors and impaired social behaviors exhibited by eIF4E transgenic mice can be reversed by the 4EGI-1, which inhibits eIF4E-eIF4G interactions and cap-dependent translation.

Reportable Outcomes

1) Poster presentation at 2011 Society for Neuroscience meeting in Washington, DC by Dr. Emanula Santini. Abstract and poster included in appendices.

2) Invited seminar, Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, Madison, WI

3) Invited speaker at Society for Neuroscience meeting, Mini-symposium entitled "Translational Control at the Synapse and in Disease", Washington, DC

4) Invited speaker at Joint Meeting of the Haifa Forum for Brain and Behavior and the Molecular and Cellular Cognition Society - Europe, University of Haifa, Haifa, Israel

5) Invited speaker at Weizmann Institute of Science - New York University Science Days: Frontiers in Brain and Cognition, Weizmann Institute of Science, Rehovot, Israel

6) Invited seminar, Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

7) Invited seminar, Intellectual and Developmental Disabilities Research Center, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA

8) Invited seminar, Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH

9) Invited seminar entitled ""Department of Neuroscience, Baylor College of Medicine, Houston, TX

10) Invited speaker at Gordon Research Conference entitled "Fragile X and Autism-related Disorders: From Basic Neuroscience to Improved Clinical Care", Stonehill College, Easton, MA

11) Invited speaker at Institute for Genomics & Systems Biology, Conte Center Brain Awareness Day public presentation, University of Chicago, Chicago, IL

12) Invited speaker at Gordon Research Conference entitled "Neurobiology of Brain Disorders: Synaptic Dysfunction and Neurodegeneration", Stonehill College, Easton, MA

Conclusion

In the first year of funding we have demonstrated that increased eIF4E expression and consequently, exaggerated cap-dependent protein synthesis results in the appearance of ASD-like behaviors in mice. We speculate that exaggerated cap-dependent protein synthesis, which is sufficient for the generation of synaptic alterations leading to ASD-like behaviors, results in enhanced translation of a specific subset of mRNAs. Importantly, our results also indicate that a pharmacological intervention that targets the formation of the eIF4F initiation complex (eIF4E+eIF4G+eIF4A) is sufficient to correct ASD-like endophenotypes displayed by the eIF4E transgenic mice. These experiments directly demonstrate that dysegulated translational control at the initiation phase of protein synthesis causes behavioral abnormalities in several domains consistent with ASD. Moreover, these results suggest that the eIF4F initiation complex is a viable therapeutic target for the treatment of individuals with ASD.

References

Hoeffer, C. A. *et al.* Removal of FKBP12 enhances mTOR-Raptor interactions, LTP, memory, and perseverative/repetitive behavior. *Neuron* **60**, 832–845 (2008).

Hoeffer, C. A. *et al.* Inhibition of the interactions between eukaryotic initiation factors 4E and 4G impairs long-term associative memory consolidation but not reconsolidation. *Proc Nat Acad Sci USA* **108**, 3383–3388 (2011).

Lewis, M. H., Tanimura, Y., Lee, L. W. & Bodfish, J. W. Animal models of restricted repetitive behavior in autism. *Behav Brain Res* **176**, 66–74 (2007).

McFarlane, H. G. *et al.* Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes Brain Behav* **7**, 152–163 (2008).

Moy, S. S. *et al.* Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav* **3**, 287–302 (2004).

Peca, J. *et al.* Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**, 437–442 (2011).

Rapin, I. & Tuchman, R. F. Autism: definition, neurobiology, screening, diagnosis. *Pediatric Clinics of NA* **55**, 1129–46, viii (2008).

Thomas, A. *et al.* Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology* **204**, 361–373 (2009).

Appendices

List of items in appendices:

1) Detailed description of behavioral paradigms
2) 2011 Society for Neuroscience abstract
3) 2011 Society for Neuroscience poster

Social Interaction Tests:

Social Approach: Mice will be placed in a three-chambered testing arena, where two areas are novel and the central area familiar to the mouse. One novel chamber will contain a caged stranger mouse (social target), the middle section the neutral starting location, and the other novel chamber identical to the first except that it does not contain a mouse (inanimate target). Time spent in each chamber will be measured and mice tested for propensity to approach and engage with a stranger mouse. ASD-like phenotypes would manifest as low social approach scores to the caged stranger mouse.

Social Novelty: This test will be conducted in the same testing apparatus as the social approach test, except that a familiar mouse will be added to the previously empty novelty chamber. In this case, preference for interaction between the familiar mouse and the stranger mouse will be tested. Under normal conditions, mice habituate to the conspecific mouse and rapidly move to investigate a novel conspecific mouse. Mice exhibiting ASD-like phenotypes may demonstrate the opposite behavior and spend more time with the familiar conspecific mouse.

Anxiety-related Behaviors:

Open Field Analysis: Mice normally remain low to the surface and near the edges of novel environments and venture to more exposed areas, i.e. the center of a space or upward by rearing, with less frequency. Measuring these parameters gives a reflection of the anxiety a mouse exhibits when exposed to a novel environment. Mice will be placed in a bright open testing arena, enclosed by transparent material. Mice will be placed in the center of the arena and allowed to explore the novel environment for 15 min. Mice with an ASD-related anxiety phenotype would be expected to spend less time in the center of the arena and show a reduced frequency to engage in rearing exploratory behaviors. Additionally, stereotypic (repetitive) movements can be captured by the tracking system used for this task, thereby allowing the measurement of this phenotype with general anxiety testing.

Elevated Plus Maze: The mice will be tested in a plus-shaped maze. Two arms are enclosed on the sides by non-transparent materials (closed) while two arms are completely exposed (open). Mice will be placed in the center of the maze and the time spent in each arm recorded. This test of anxiety and exploratory behavior (novelty) will measure the tendency of the mouse to leave the “protected” closed arms and venture into the exposed area in the open arms of the maze. ASD individuals generally display enhanced anxiety. If the eIF4E transgenic mice display phenotypes similar to those in ASD, then they would display reduced time in the open arms of the maze.

Repetitive/Perseverative Behaviors:

Marble Burying: Mice often “bury” or otherwise conceal glass marbles placed in their home cages. Burying behavior is thought to involve reward pathways associated with either anxiety relief or

compulsiveness, and is considered an animal model of anxiety and obsessive-compulsive disorder (OCD), but also has been employed in the examination of ASD mouse models. Increased number of marbles buried by eIF4 transgenic mice might reflect enhanced anxiety or a tendency to exhibit repetitive behavior.

Arm Reversal in Y-Maze: Mice will be trained in a simple Y-water maze based escape task. Visual cues are located at the either arm of the Y-maze. Mice will be trained to locate a submerged escape platform in one arm denoted by a specific visual cue. This version of the Y-maze does not require food restriction to enforce appetitive acquisition. Then the location of the platform will be changed. The ability of the mice to change their arm choice will be measured. In this way, resistance to change (perseveration) can be observed by measuring the latency to choose the new arm compared to the original escape location. This task will permit us to more specifically separate perseverance phenotypes from phenotypes derived from search strategies. This task models the propensity for ritualistic and repetitive behaviors exhibited by ASD-afflicted individuals. Mice with an ASD-like phenotype would be expected to display an increase in the time spent investigating previous escape locations.

Prepulse Inhibition: This test measures the hearing and reflex startle response of the animals. The animal will be removed from its home cage and is place into a sound proof chamber. The startle response tone (120 decibels for 20 ms) will be given and startle response scored by an automated system. Then a series of mild prepulse tones will be paired with the 120 decibel tone and the response scored. The prepulse tones will be 74, 78, 82, 86, and 90 decibels and 20 ms in duration. Each prepulse tone will be paired with the 120 decibel tone, with the prepulse tone increasing with each pairing. Four trials of each pairing will be performed. The startle response decreases with increasing prepulse tone in wild-type mice.

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NEUROSCIENCE 2011

Presentation Abstract

Program#/Poster#: 150.02/R10

Presentation Title: eIF4E transgenic mice as a novel animal model of autism spectrum disorders.

Location: Hall A-C

Presentation time: Sunday, Nov 13, 2011, 9:00 AM -10:00 AM

Authors: ***E. SANTINI**, E. KLANN;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Autism spectrum disorders (ASDs) are a heterogeneous group of heritable neuropsychiatric disorders whose symptoms, which include abnormal social interaction, impaired communication, and repetitive/perseverative behaviors, appear in early childhood and continue throughout life. One of the leading hypotheses for a common molecular mechanism underlying ASDs is alteration in the translational control machinery. In particular, studies of humans and evidence obtained from animal models have revealed that upregulated mTORC1 signaling is a molecular abnormality that may contribute to ASDs-like behaviors. Consistent with this notion, it recently was found that at least two autistic patients carry a mutation in the promoter region of the EIF4E gene, which encodes the cap-binding translation factor eIF4E, a downstream effector of the mTORC1-signaling pathway. Importantly, in vitro studies have demonstrated that this mutation increases eIF4E promoter activity, suggesting that some these autistic patients may have increased expression of eIF4E.

eIF4E mediates cap-dependent translation initiation by binding eIF4G, thereby forming the eIF4F initiation complex. Activation of the mTORC1 signaling pathway promotes protein synthesis by releasing eIF4E from its repressor eIF4E-binding protein (4E-BP), thus increasing the availability of eIF4E to interact with eIF4G.

We recently have began studies of a transgenic mouse line overexpressing eIF4E to test the hypothesis that increased protein synthesis is a causative factor in ASDs-like behaviors. We have found that eIF4E transgenic mice exhibit increased eIF4F complex formation as measured by the interaction between eIF4E with

eIF4G, suggesting increased protein synthesis. The eIF4E transgenic mice also exhibit ASDs-like behaviors, including repetitive and stereotypical behaviors and impairments in reversal learning, suggesting behavioral inflexibility. In addition, the eIF4E transgenic exhibit alterations in several forms of synaptic plasticity that are due to excessive cap-dependent protein synthesis. Thus, eIF4E mice are a new resource that can be utilized to specifically address the involvement of dysregulated translational control in ASDs.

Disclosures: **E. Santini:** None. **E. Klann:** None.

Keyword(s): PROTEIN SYNTHESIS

AUTISM

ANIMAL MODEL

Support: Department of Defense, MRMC AR100216

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eIF4E transgenic mice as a novel animal model of autism spectrum disorders

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150.02/R10

Introduction

Autism spectrum disorders (ASDs) are a heterogeneous group of heritable neuropsychiatric disorders whose symptoms, which include abnormal social interaction, impaired communication, and repetitive/perseverative behaviors, appear in early childhood and continue throughout life. One of the leading hypotheses for a common molecular mechanism underlying ASDs is alteration in the translational control machinery. In particular, it seems that upregulated mTORC1 signaling is a molecular abnormality that may contribute to ASDs-like behaviors. Consistent with this notion, it was recently found in autistic patients a mutation increasing *eIF4E* promoter activity.

eIF4E mediates cap-dependent translation initiation by binding eIF4G, thereby forming the eIF4F initiation complex. Activation of the mTORC1 signaling pathway promotes protein synthesis by releasing eIF4E from its repressor eIF4E-binding protein (4E-BP), thus increasing the availability of eIF4E to interact with eIF4G.

Here we employed a transgenic mouse line, in which the expression of eIF4E is augmented, to test the hypothesis that increased cap-dependent protein synthesis play a causative role in the etiology of ASDs.

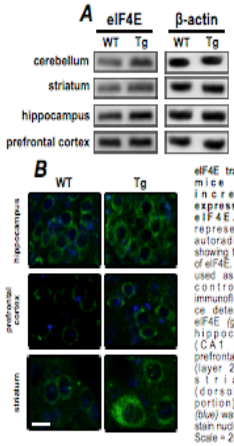
Materials and Methods

Conclusions

We show that eIF4E is overexpressed in the brain of the transgenic mice without compensation by other translational control proteins. We have also found that eIF4E transgenic mice exhibit increased eIF4F complex formation as measured by the interaction between eIF4E with eIF4G, suggesting increased protein synthesis. Importantly, eIF4E transgenic mice exhibit some specific ASDs-like behaviors, such as repetitive and stereotypical behaviors and impairments in reversal learning, suggesting behavioral inflexibility. In addition, eIF4E transgenic exhibit alterations in several forms of synaptic plasticity that are due to excessive cap-dependent protein synthesis. Thus, eIF4E mice are a new resource that can be utilized to specifically address the involvement of dysregulated translational control in ASDs.

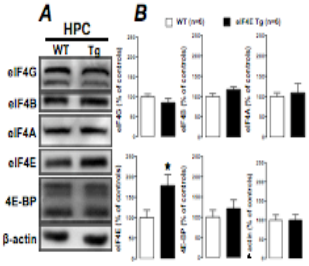
Results

1 eIF4E is ubiquitously overexpressed in the brain of eIF4E transgenic mice



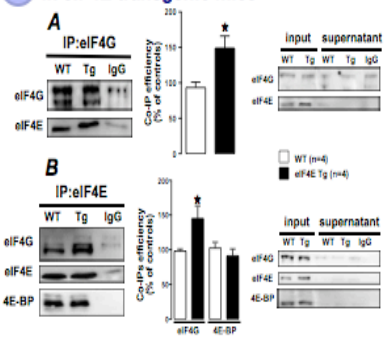
eIF4E transgenic mice show increased expression of eIF4E. (*A*) representative autoradiograms showing the levels of eIF4E, β-actin is used as loading control; (*B*) immunofluorescence detection of eIF4E (green) in hippocampus (CA1 area), prefrontal cortex (layer 2/3) and striatum (dorsolateral portion). DAPI (blue) was used to stain nuclei. Scale = 20 μm

2 Basal expression of translational control proteins is not altered in eIF4E transgenic mice



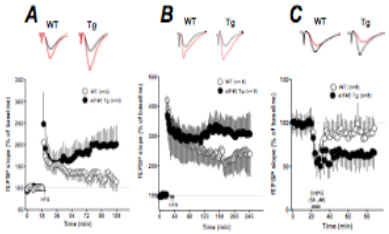
Expression of translational control proteins is not perturbed by increased eIF4E levels in eIF4E transgenic mice. (*A*) representative autoradiograms showing the total levels of translational control proteins. β-actin is used as loading control; (*B*) Data are expressed as percentage of wild-type and represent means ± SEM. Levels of eIF4E are significantly higher in eIF4E transgenic mice as compared to wild type littermates. t-test, *p<0.05.

3 eIF4E is preferentially bound to eIF4G in eIF4E transgenic mice



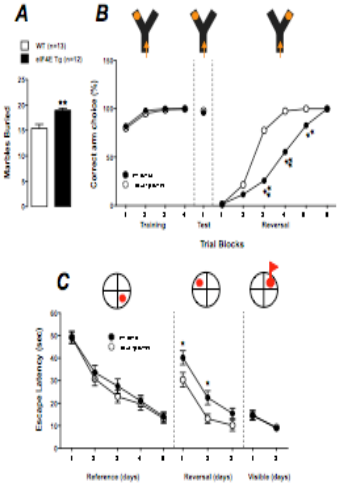
Overexpression of eIF4E leads to increased formation of the initiation complex eIF4F. eIF4G (*A*) and eIF4E (*B*) were immunoprecipitated (IP) using hippocampal protein extracts of wild-type and eIF4E transgenic mice. Representative autoradiograms showing eIF4G (*A*, right) and eIF4E (*B*, right) co-immunoprecipitated (co-IP) with theirs binding partners; Bar graphs of data expressed as percentage of the ratio eIF4E/eIF4G (*A*, center) or eIF4G/eIF4E and 4E-BP/eIF4E (*B*, center) and represent means ± SEM. *t*-test, *p<0.05. Representative autoradiograms showing control parameters of IP experiments (*A*, *B*, left).

4 eIF4E transgenic mice display altered hippocampal synaptic plasticity



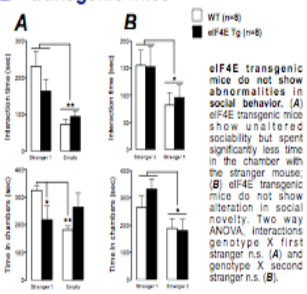
eIF4E transgenic mice exhibit increased E-LTP, augmented L-LTP and enhanced mGluR-LTD. Representative fEPSP of hippocampal slices of eIF4E transgenic mice and their wild-type littermates that receive one train of 100 Hz HFS for 1 sec (*A*, upper), four trains (5min interstrain interval) of 100 Hz HFS for 1 sec (*B*, upper) or application of DHPG (50 μM, 10 min; *C*, upper). Black traces: baseline, red traces: poststimulation; (*A*) E-LTP induced by one train HFS is increased in Tg mice, p<0.05 RM-ANOVA; (*B*) L-LTP induced by four trains of HFS is enhanced in Tg mice, p<0.05 RM-ANOVA; (*C*) mGluR-LTD induced by bath application of DHPG is increased in eIF4E Tg mice, p<0.005 RM-ANOVA.

5 eIF4E transgenic mice show perseverative and repetitive behavior



eIF4E transgenic mice exhibit enhanced repetitive behavior in three different paradigms. (*A*) eIF4E transgenic mice showed increased marble burying behavior, *t*-test **p<0.01; (*B*) eIF4E transgenic mice exhibit impaired reversal Y-maze test, ***p<0.001 and **p<0.01; (*C*) eIF4E transgenic mice show deficits in MWM's reversal learning, *p< 0.05. Repeated measure ANOVA followed by Bonferroni-Dunn test. A significant interaction was found between genotype and time; (*B*) $F_{(3,69)}$=43.27, p<0.0001], [(*C*) DAY1: $F_{(3,69)}$ =40.34; p<0.05, DAY2: $F_{(3,69)}$=45.78, p<0.05, DAY3: n.s.]

6 Social behavior of eIF4E transgenic mice



eIF4E transgenic mice do not show abnormalities in social behavior. (*A*) eIF4E transgenic mice show unaltered sociability but spent significantly less time in the chamber with the stranger mouse; (*B*) eIF4E transgenic mice do not show alteration in social novelty. Two way ANOVA, interactions genotype X first stranger n.s. (*A*) and genotype X second stranger n.s. (*B*).

Acknowledgments

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Supporting Data

All figures and figure legends were embedded in the Body section of the report.